IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Ari HINKKANEN

Serial No.: 09/015,399 Examiner: M. Lubet

Filed: 29 January 1998 Group Art Unit: 1644

For: A NEW FUSION PROTEIN AND ITS USE IN AN IMMUNOASSAY FOR THE SIMULTANEOUS DETECTION OF AUTOANTIBODIES RELATED TO INSULIN-DEPENDENT DIABETES MELLITUS

DECLARATION UNDER 37 C.F.R. § 1.131(a)

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Ari Hinkkanen, the applicant for the above-identified patent application, declare as follows:

1. That some time on or prior to 22 August 1996, I conceived the idea of using fusion proteins comprising insulin, glutamic acid decarboxylase 65 (GAD65) and/or ICA 512 (IA2) for use in an assay to determine whether a person has juvenile or type 1 diabetes (IDDM).

2. The conception of the present invention and eventual reduction to practice occurred in Finland.

3. The date of the conception was determined by the computer record of Exhibit 1 which is a printout of said computer drafted document. The file was created with Microsoft Word and the computer shows a "last modified" date of 22 August 1996. Furthermore, the document of Exhibit 1 was created specifically for a confidential meeting which was held on 26 August 1996 between Applicant, colleagues of Applicant and Orion Diagnostica. Exhibit 2 is a Confidentiality Agreement drafted for the meeting listing the 26 August 1996 date. This Confidentiality Agreement was never signed although the meeting did take place as scheduled, with all persons named in the Confidentiality Agreement attending the meeting. The Confidentiality Agreement shown as Exhibit 2 was created with Microsoft Word and the computer shows a "last modified" date of 23 August 1996 for this document.

4. Exhibit 3 is an English translation of Exhibit 1, the translated Exhibit 3 being prepared on May 12, 2000. Page 1 of Exhibit 3 indicates that the document concerns methods for predicting whether a person has IDDM and that the method uses islet cell antigens. Included in the list of islet cell antigens are GAD65, IA2 and insulin. The document details some of the methods for testing for IDDM used at the time the document was prepared and discusses some of the problems of those prior art methods. Page 6 of the English translation sets forth the idea of using a fusion protein comprising different ICA proteins, an example being given of using a GAD65/ICA512/38kD fusion protein.

5. It is further declared that the accompanying exhibit may not be a complete record of applicant's data concerning the invention of the instant patent application and is not necessarily meant to represent the earliest date of conception. The accompanying exhibits are presented solely to prove conception of the invention prior to the date of the Borg et al. and the Wiest-Ladenburger et al. references cited as prior art.

The declarant further states that the above statements were made with the knowledge that willful false statements and the like are punishable by fine and/or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that any such willful false statement may jeopardize the validity of this application or any patent resulting therefrom.

Dated: May 22, 2000

Ari Hinkkanen

EXHIBIT 1

CERTIFICATE OF VERIFICATION

I, Ari Hinkkanen, of Turku, Finland, state that the attached document is a true and complete translation to the best of my knowledge of Exhibit 1 which was submitted together with a Declaration Under 37 C.F.R. § 1.131(a) on 25 May 2000 during the prosecution of Serial No. 09/015,399. The original document (Exhibit 1) is written in the Finnish language. I performed the translation of Exhibit 1 into the attached English language document. I am proficient in both the Finnish and English languages.

Dated this 18 day of August, 2000

Signature of translator: _____

Ari Hinkkanen

NUORUUSAJAN DIABETEKSEN (IDDM) ENNUSTUSMENETELMÄT

SAAREKESOLU-ANTIGEENIT:

- *GLUTAMIC ACID DECARBOXYLASE 65, GAD65 (KARLSEN ET AL. PNAS 1991)*

- *ISLET CELL ANTIGEN ICA512 (IA2) (RABIN ET AL. J IMMUNOL 1994)*

- *P69 (DOSCH ET AL. , PIETROPAOLO ET AL. J CLIN INVEST 1993.)*

- *INSULIINI*

- *GLIMA 38 (AANSTOOT ET AL . J AUTOIMM 1996)*

- *MUUT*

GAD65

TÄLLÄ HETKELLÄ INFORMATIIVISIN JA AINOA, JOKA ON KÄYTÖSSÄ OULUSSA!

- TESTI TEHDÄÄN IN VITRO TRANSKRIBOIDULLA JA TRANSLOIDULLA, RADIOAKTIIVISESTI (35S) LEIMATULLA GAD65 PROTEIINILLA IMMUNOPRESIPITAATIOON PERUSTUEN (PROTEIN-A SEPHAROSE)

- TYÖLÄS
- ANTIGEENI VANHENEE
- TARVITAAN RADIOISOTOOPPIA

ICA512

ICA-512 ON INFORMATIIVINEN

- OMAA MAHDOLLISESTI FOSFATAASIAKTIIVISUUTTA
- VAIN INTRASELLULAARIOSA OMAA RELEVANTTEJA EPITOOPPEJA
- TUOTETTU GAD65 TAVOIN IN VITRO-TRANSLAATIOLLA (ICA512BDC: KAWASAKI ET AL. SUBMITTED)

P69

INFORMATIIVISUUDESTA RISTIRIITAISIA TULOKSIA

- HUONOLIUKOINEN, EI SAADA LIUOTETTUA ILMAN KAOTROOPPEJA
- TUOTETTU E.COLISSA JA PUHDISTETTU METALLIKELAATTI- AFFINITEETTIKROMATOGRAFIALLA

GLIMA 38

- INFORMAATIOARVO EI VIELÄ TARKALLEEN TIEDOSSA; (19/86 POSITIIVISTA IDDM, 0/65 KONTR; AANSTOOT ET AL. 1996)

- AMFIFIILINEN MEMBRAANIPROTEIINI (AIVOT, SAAREKESOLUT)
- VAIKEALIUKOINEN GLYKOPROTEIINI (22KDA)
- KLOONI SAATAVILLA?

INSULIINI

IAA ASSAY: NESTEFAASISSA TAPAHTUVA KOMPETITIOON PERUSTUVA RADIO-ASSAY; VARDI ET AL. DIABETES 1987

SAAREKESOLU-/KUDOSNÄYTE (KONFOKAALIMIKROSKOPIA)

INFORMATIIVISUUS (AANSTOOT ET AL.)

IDDM POTILAAT N=130

38KD+	22/130	17%
GAD65+	98/130	75%

$38KD^{NEG}/GAD65^{NEG}$ 24%, JOISTA 9 $ICA512^{POS}$., YHT. 91% POSITIIVISIA JOLLEKIN NÄISTÄ KOLMESTA.

PREDIABEETIKOT N=44

GAD65	33/44	75%
GAD65+38KD	35/44	80%
ICA512+GAD65	37/44	84%
ICA512+38KD	22/44	50%

PREDIKTIOTESTIN KEHITYSNÄKYMÄT:

MITÄ TARVITTAISIIN?

ICA AUTOVASTA-AINEIDEN MÄÄRITYSMENETELMÄ, JOKA ON

- KATTAVA
- LUOTETTAVA
- YKSINKERTAINEN
- NOPEA
- HALPA
- SUURILLE MÄÄRILLE
- (EI-RADIOAKTIIVINEN)

MITÄ ON TEHTY TAI MENEILLÄ?

- GAA-RADIOTESTI TOIMII TÄLLÄ HETKELLÄ HYVIN OULUSSA

- GAD65 ON TUOTETTU BACULO-SYSTEEMISSÄ, LIUKOISUUSONGELMIA ON OLLUT, TESTIÄ EI OLE KEHITETTY

- IMMUNOAFFINITEETTI-PUHDISTUS TOIMII JOTENKIN, ONGELMANA EPITOOPPIEN SÄILYMINEN

- DELEETIOITA ON TEHTY GAD65:EEN, VASTAAVAT BACULOVIRUKSET ON OLEMASSA, MUTTA PROTEIINEJA EI VIELÄ TUOTETTU LAAJASSA SKAALASSA

- BHK21-SOLUISSA YRITETTY TUOTTAA GAD65, MUTTA EKSPRESSIO LIIAN ALHAINEN

- P69 ON TUOTETTU BACULOSYSTEEMILLÄ; KAKSI C-TERMINAALISTA DELEETIOTA JA TÄYSPITKÄ KAIKKI LIUKENEMATTOMIA, UUSI HIS-KONSTRUKTI TEKEILLÄ

- ICA512 SISÄLTÄVÄ BACULOGENOMI TEHTY, MUTTEI TUOTA VIRUSTA

- METALLIKELAATTI-PUHDISTUS ON PYSTYTETTY JA TOIMII MUILLA (ESIM. AIVO-) ANTIGEENEILLÄ

SUUNNITTEILLA

- TUOTETAAN LAAJASSA MITTAKAAVASSA ANTIGEENISESTI AKTIIVISTA ICA-PROTEIINIA ESIM. BACULOVIRUS-SYSTEEMILLÄ (FLAG-EPITOOPPI; HIS-HÄNTÄ)

- KEHITETÄÄN GAD65 TESTI 96-KUOPPALEVYLLE FLUORESENSSI-DETEKTIOLLA (ESIM. TR-FIA)

- KEHITETÄÄN ICA512:LLE VASTAAVA PUHDISTUSMENETELMÄ JA TESTI

- SAMOIN MUILLE, MAHD. AIVAN UUSILLE ICA:LLE (GLIMA 38 = 38KD)

- MITATAAN VASTEET YKSITTÄISILLE PROTEIINEILLE YHDESSÄ KUOPASSA KÄYTTÄMÄLLÄ ERI LEIMOJA

- VALMISTETAAN FUUSIOPROTEIINI, JOSSA FLAG EPITOOPPI SEKÄ FLEKSIIBELIEN LINKKERIEN VÄLITYKSELLÄ YHDISTYVÄT ERI ICA- PROTEIINIEN INFORMATIIVISET EPITOOPIT

  ESIM.: GAD65/ICA512/38KD (78/86 = 91% POTILASTA POSIT. JOLLEKIN NÄISTÄ KOLMESTA; AANSTOOT ET AL. 1996)

MITÄ FASILITEETTEJA TOIMINTA EDELLYTTÄÄ?

- REKOMBINANTTI-ICA-ANTIGEENIEN TUOTTOYKSIKKÖ (BACULO-, SFV-VIRUS, MUUT)

- SOLUKASVATUSYKSIKKÖ

- PROTEIINIANALYTIIKKA-YKSIKKÖ

- FLUOROMETRIA-YKSIKKÖ

- NÄYTTEIDEN TOIMITUS

- TEKIJÄT

Translation in English (12.05.2000)

Prediction m thods for juvenile or type 1 diabetes (IDDM)

Islet cell antigens:

- Glutamic acid decarboxylase 65, GAD65 (Karlsen et al. 1991, PNAS)
- Islet cell antigen ICA 512 (IA2)(Rabin et al. J Immunol 1994)
- p69 (Dosch et al., Pietropaolo et al. J Clin invest 1993)
- Insuliini
- Glima 38 (Aanstoot et al. J Autoimm. 1996)
- Others

GAD65

At the moment the most informative test and the only one, which is in use in Oulu

The t st is made using in vitro transcrib d and translated, radioactively labeled GAD65 protein and based on immunoprecipitation (Protein A Sepharose)

- laborous
- antigen decays
- radioisotopes needed

ICA512

ICA-512 is informative

- possesses possibly phosphatase activity
- onli intracellular part has informative epitopes
- produced like GAD65 via in vitro translation (ICA512BCD: Kawasaki et al. submitted)

p69

Contradicory results on informativity

- poorly soluble, can not be solubilized witout chaotrops
- produced in E. coli and purified with metal chelate affinity chromatography

Glima 38

- informativity not clear yet (19/86 positive of IDDM patients, e/65 of controls, Aanstoot et al. 1996)
- amphiphilic membrane protein (brain, islet cells)
- poorly soluble glycoprotein (22 kDa)
- clone available?

Insulin

IAA assay: Radio assay which is done in liquid phase and is based on competition; Vardi et al. Diabetes

Islet cell/tissue sample (Confocal microscopy)

Informativity (Aanstoot t al.)

IDDM patients N = 130

38kD+	22/130	17%
GAD65+	98/130	75%

38kDneg/GAD65neg 24%, of which 9 ICA512pos, together 91% positive for one of the three antigens.

Prediabetic individuals N = 44

GAD65	33/44	75%
GAD65+38kD	35/44	80%
ICA512+GAD65	37/44	84%
ICA512+38kD	22/44	50%

Visions for development of the prediction test:

What is needed?

Determination method for ICA autoantibodies which is:

- covering
- reliable
- simple
- rapid
- inexpensive
- for large sample numbers
- non-radioactive

What has been done or is ongoing?

- GADA radio test works well in Oulu at the moment

- GAD65 has been produced in baculo system, problems appeared with solubility, test not yet developed

- immunoaffinity chromatography works fairly, problem is conservation of the epitopes

- deletions done in GAD65, corresponding baculoviruses exist, but proteins have not yet been produced in large scale

- attempts were made to produce GAD65 in BHK21 cells, but expression levels too low

- p69 has been produced with baculo system, two C terminal deletion proteins and the full-length protein insoluble; new his-construct in preparation.

- ICA512 baculo genome has been made, but does not produce virus

- metal chelate chromatography has been set up and works with other, e.g brain antigens

Under planning

Antigenically active ICA proteins are produced in large scale .g. with baculo system (flag epitop , his-tail)

- GAD65 test for 96 well microtiter plate will be developed with fluorescence detection (e.g. TR-FIA)

- corresponding purification and test will be developed for ICA512

- same is valid for other, quite new ICA:s (Glima 38 =38 kD)

- the responses will be measured for individual proteins in one well using different labels

- a fusion protein will be prepared with flag epitope, and flexible linker to combine the informative epitopes from different ICA proteins

for example: GAD65/ICA512/38kD (78/86=91% of patients positive for one of these three; Aanstoot et al. 1996)

What facilities does the activity necessitate?

- production unit for recombinant antigens (baculo-, SF-virus, others)
- cell culture unit
- protein analysis unit
- fluorometry unit
- organization of samples
- the executors

EXHIBIT 2

A

CONFIDENTIALITY AGREEMENT

This agreement, effective August 26th,1996 by and between professor Olli Simell, professor Mikael Knip, professor Timo Lövgren, docent Jorma Ilonen, docent Ari Hinkkanen and Dr. Tuula Simell (RESEARCHERS) and Orion Corporation, ORION DIAGNOSTICA (ORION), having an address at Koivumankkaantie 6, SF 02200 Espoo, Finland, both inclusive of their affiliates, successors, etc., shall govern the CONFIDENTIAL INFORMATION exchanged in writing or orally between RESEARCHERS and ORION in connection with the discussions started on August 26th, 1996 .

CONFIDENTIAL INFORMATION is non-public technical information related to the research project of RESEARCHERS aiming at DIabetes Prediction and Prevention (DIPP) and to business development measures of ORION aiming at early detection of prediabetic individuals through in-vitro diagnostic laboratory testing
, and non-public information disclosed by either partner during discussions related to future licensing and/or collaborative agreements between RESEARCHERS and ORION.

With regard to CONFIDENTIAL INFORMATION , the receiving partner RESEARCHERS or ORION hereby agrees not knowingly to use or disclose CONFIDENTIAL INFORMATION to others (except to its employees or agents who reasonably require some for the purpose hereof and who are bound to it by like obligations as to confidentiality), except that the receiving partner shall not be prevented from using or disclosing information :

(I) which the receiving partner can demonstrate by written records was known to receiving partner prior to the date of disclosure hereunder; or

(II) which is now or becomes in the future, public knowledge other than by breach of this Agreement by receiving partner; or

(III) which is lawfully obtained by the receiving partner from a source independent of the supplying partner RESEARCHERS or ORION; or

(IV) which is subsequently developed by the receiving partner independent of the CONFIDENTIAL INFORMATION received hereunder; or

(V) which is disclosed by the disclosing partner to any third party on a non-confidential basis

It is agreed that the receipt of CONFIDENTIAL INFORMATION by the receiving partner shall not be deemed an admission by the receiving partner of the novelty or patentability of said CONFIDENTIAL INFORMATION.

It is further agreed that the partners do not commercially exploit such CONFIDENTIAL INFORMATION without written consent of another partner.

The obligations of both partners under the terms of this Agreement shall remain in effect for five (5) years from the effective date hereof.

This Agreement shall be interpreted and enforced in accordance with the laws of Finland.

Date: 1996

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Orion Corporation
ORION DIAGNOSTICA
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